Improving Estimates of Contaminant Exposure for Mobile Organisms: An Assessment of Area-weighted Home Range Exposure Estimates Applied to the Relationship Between Sediment Chemistry and Liver Lesions in English Sole

Jeffrey H. Stern
King County Dept. Natural Resources and Parks

Dan P. Hennessy *and* ***Clay R. Patmont***
Anchor Environmental, L.L.C.

Abstract
Liver lesions in English sole (*Pleuronectes vetulus*) have been identified as biomarkers for exposure to polycyclic aromatic hydrocarbons (PAHs). Federal researchers have been investigating the correlation between PAHs and the prevalence of liver lesions in these fish from sites along the West Coast, including Puget Sound, and proposing the relationship be used to develop sediment quality benchmarks. Appropriate characterization of exposure of these mobile organisms to PAHs is essential to developing such a relationship. Area-weighted home range PAH exposure estimates for English sole collected in Puget Sound were developed and compared with existing data. Spatially and temporally related chemistry and lesion data from urban and background areas were compiled for areas with enough sediment data to quantify spatial variability. Area-weighted average PAH concentrations were calculated using Thiessen polygons. Exposure estimates for urban embayments were 2 to 5 times greater than those estimated using only the synoptic trawl line samples. Proposed sediment quality benchmarks derived by non-linear "hockey-stick" regression were 2 to 3 times greater using the area-weighed home range exposure estimates. Results verified the relationship between sediment chemistry and liver lesions but the difference in the results using a more robust statistical approach suggest an improved method for calculating exposure estimates for mobile organisms. Such exposure estimates are critical to risk assessments and biological opinions needed by decision-makers to make resource decisions.

Introduction
Lesions in the livers of English sole (*Pleuronectes vetulus*) have been identified as biomarkers for exposure to polycyclic aromatic hydrocarbons (PAHs) in marine and estuarine sediment. Using synoptic sediment chemistry and hepatic (liver) lesion data collected from sites along the West Coast, including several sites in Puget Sound, researchers with the National Oceanic and Atmospheric Administration (NOAA) have been investigating the correlation between PAHs and the development or prevalence of liver lesions in these fish (Horness et al. 1998; Johnson 2001; Johnson et al. 2002). Additional studies conducted in Puget Sound by the Washington Department of Fish and Wildlife (WDFW) have also documented this PAH correlation with lesion prevalence (O'Neill et al. 1999). NOAA has proposed using a non-linear hockey stick regression model to define a threshold sediment concentration where lesion prevalence should increase above background incidence levels (Horness et al. 1998; Johnson 2001; Johnson et al. 2002).

The hockey stick regression model is one of several dose-response relationships that can be used to assess carcinogenic effects of chemicals. In all these relationships, the response (some measure of cancer risk) is equal to the exposure times the duration. In this case, both the response and the duration are directly measured by sampling the fish (lesion prevalence and age, respectively). The methods and sample size used to produce estimates of lesion prevalence and age provide good estimates of each of these variables in the environment. However, exposure of the English sole to the chemicals of concern (i.e., PAHs) can not be directly measured in the field. Therefore, a critical element of the field data-based predictive relationship is the exposure estimate. At present, the sediment concentration is being used as a surrogate for actual exposure. Although several factors control bioavailability of chemicals in sediments, for the purposes of this study, this assumption was accepted based on the demonstrated correlation of sediment concentration to lesion prevalence. Since English sole are a mobile species, the range of sediment any individual is exposed to becomes a significant factor in the exposure estimate. This study investigates a method to improve the estimate of exposure from sediments for mobile organisms.

Sediment exposure estimates used by NOAA in the initial development of the benchmarks were based on limited sediment sampling data collected across the trawl-line, typically resulting in three sediment samples per fish trawl (Horness et al. 1998). Because of the inherent spatial variability of sediment quality measurements in the Puget Sound region, use of such limited sediment sampling data leads to a high degree of uncertainty in individual sediment exposure estimates. Moreover, characterization of sediment exposure only within the immediate trawl-line area is not consistent with the documented home-range behavior of English sole, which, although demonstrating "pronounced homing" during seasonal spawning migrations, have been shown to move several kilometers or more over short periods in open water environments (Day 1976). Moreover, a much more extensive set of sediment chemistry data are now available within some of the fish trawl sampling areas, owing to detailed ambient monitoring programs and site-specific (e.g., Superfund) investigations. Multiple samples can be used at some locations to lower uncertainty of exposure estimates for an area that English sole are likely to forage during their seasonal residence. Considerable liver lesion data have also been collected by NOAA and the Washington Department of Fish and Wildlife (WDFW) from Puget Sound that were not included in the initial data set (Myers et al. 2001; WDFW 2002). An analysis similar to Horness et al. (1998) and Johnson et al. (2002) was conducted selecting from all available Puget Sound data and incorporating a reasonable minimum home range exposure area appropriate for English sole. The goal of this work is to identify a more accurate method to estimate exposure from sediments for mobile organisms and to illustrate issues that should be addressed prior to defining a threshold sediment concentration that may be used for environmental management decisions.

Methods

Using the work by NOAA (Horness et al. 1998; Johnson 2001; Johnson et al. 2002) as a starting point, we investigated the relationship between home-range-based area-weighted exposure estimates and lesion data for English sole. The work presented here is a regional evaluation focusing on Puget Sound sites where PAHs have been identified by the regulatory agencies (U.S. Environmental Protection Agency [EPA] and Washington Department of Ecology [Ecology]) as primary chemicals of potential concern. As generally discussed in Johnson et al. (2002), because of the potential for interactive effects among co-occurring compounds, sites were selected for this analysis that contained relatively low levels of other carcinogenic or mutagenic compounds. For example, sediment sites where EPA and/or Ecology have identified polychlorinated biphenyls (PCBs) as the primary chemical of potential concern (e.g., Duwamish Waterway) were excluded from this analysis. O'Neill et al. (1999) demonstrated that PCBs can account for some of the observed relationship between lesion prevalence and sediment chemistry. Excluding such PCB sites minimizes interactive effects of other compounds that could be argued to contribute to the correlation. Background stations were selected based on the availability of liver lesion data and historical use by EPA, Ecology, and NOAA as reference sites for environmental management decisions and inclusion in the NOAA analysis.

Spatially and temporally related sediment chemistry and fish lesion data in Puget Sound were compiled. The locations used in this study are shown in Figure 1. Area-weighted average sediment PAH concentrations were calculated from Thiessen polygons using a geographical information system (GIS) platform. Thiessen polygons were determined for all the station locations within a given home range area and the area of each polygon was used to weight the calculated average sediment concentration. The calculated home range exposure estimates were paired with English sole liver lesion data. The relationship between home range exposure and incidences of liver lesions was evaluated using the non-linear "hockey-stick" regression procedure described in Horness et al. (1998) and Johnson et al. (2002). The following sections provide details on the methods used to conduct this work.

Data Compilation

Sediment chemistry and liver lesion data for Puget Sound were collected from numerous sources. Table 1 summarizes the data available at the locations used for this analysis.

All of the sediment chemistry data listed in Table 1 were collected and analyzed using current Puget Sound Estuary Program (PSEP) protocols, incorporating independent data validation in accordance with EPA and Ecology requirements. Ecology and/or EPA have deemed sediment data included in the reports listed above as suitable for site characterization purposes.

Liver lesion data were compiled from NOAA studies (Collier et al., 1997; NOAA, 1998) and WDFW monitoring studies in Puget Sound (WDFW, 2002). Liver lesion data were not independently evaluated for QA/QC. However, these NOAA and WDFW reports indicated that all data utilized in this analysis were collected in accordance with current NOAA protocols, as described in Johnson et al. (1992) and Collier et al. (1997).

Sediment Chemistry Data: Only surface sediment chemistry data (collected from within 10 cm of the mud line) were used for this evaluation, and were limited to high molecular weight PAH (HPAH) determinations. HPAHs were selected and summed according to the Washington State Sediment Management Standards (SMS) (WAC 173-204), and included the following ten chemicals, including those identified with the greatest potential for carcinogenicity:

Figure 1. Locations with paired English sole hepatic lesion and sediment chemistry data.

- Benzo[a]anthracene
- Benzo[b]fluoranthene
- Benzo[k]fluoranthene
- Benzo[g,h,i]perylene
- Benzo[a]pyrene
- Chrysene
- Dibenzo[a,h]anthracene
- Fluoranthene
- Indeno[1,2,3-cd]pyrene
- Pyrene

As discussed in Johnson et al. (2002), HPAHs are generally recognized as most closely associated with carcinogenicity and, assumedly, the induction of liver lesions in flatfish. HPAHs also have a higher affinity to organic or inorganic material and persist in sediments compared to the more soluble and biodegradable low molecular weight PAHs. Although other PAH compounds have been identified that also exhibit mutagenic properties, HPAH include the most potent mutagenic compounds (Larsen and Larsen 1998; NTP 1998) and tend to account for most of the PAH potency found in sediment samples (usually >90-95 percent of the cumulative cancer risk or potency quotient), particularly in urbanized areas that are dominated by industrial sources and combustion products. While there are other PAH compounds that

Table 1. Biological and Chemical Data From Puget Sound Used in Analysis of Areal-Weighted Home Range Averaging and Liver Lesions for English Sole

Site	Data Available	Data Source	Collection Dates
Hylebos Waterway	Liver Lesion	Collier et al. 1997	1994-1995
	Sediment Chemistry	SEDQUAL, 2002	1994-1996
Eagle Harbor	Liver Lesion	WDFW, 2002	1991, 1998-1999
	Sediment Chemistry	CH2MHill, 1988	1988
		SAIC, 1998	1997
		PSAMP, 2001	1998
		Striplin, 2000	1999
Elliott Bay	Liver Lesion	WDFW, 2002	1989-1994
		NOAA, 1998	1984-1991
	Sediment Chemistry	SEDQUAL, 2002	1983-1992
Thea Foss Waterway	Liver Lesion	WDFW, 2002	1997
	Sediment Chemistry	Hart Crowser, 1999	1997
Carr Inlet	Liver Lesion	WDFW, 2002	1996
	Sediment Chemistry	PSAMP, 2001	1998
Colvos Passage	Liver Lesion	Collier et al. 1997	1994-1995
	Sediment Chemistry	PSAMP, 2001	1998
Nisqually Delta	Liver Lesion	WDFW, 2002	1993, 1995-1999
		NOAA, 1998	1984-1986, 1990-1991
	Sediment Chemistry	NOAA, 1998	1984-1986, 1991
		PSAMP, 2001	1998

exhibit relatively high potency, mostly PAH derivatives, these compounds are not usually found at detectable levels in environmental samples and have not been routinely analyzed in Puget Sound sediments. Moreover, because of strong correlation among individual HPAHs (e.g., see Johnson et al. 2002), the 10 HPAHs listed above provide a reasonably consistent measure of total HPAH exposure while allowing for use of the large base of sediment samples collected by other studies and direct comparison with empirically-based sediment standards such as SMS.

Liver Lesion Data: Liver lesion data were obtained from NOAA (Collier et al. 1997; NOAA, 1998) and WDFW (2002). The NOAA lesion data were categorized by lesion type; for this analysis, neoplasms; preneoplastic foci of cellular alteration (FCA), which could be precursors of neoplasms; and/or specific degeneration/necrosis (SDN) were used to define a lesion occurrence. The WDFW (2002) lesion data were presented as "hit" or "no hit". Consistent with Johnson et al. (2002), a hit defined by WDFW included: neoplasia, FCA, or SDN lesion types. The lesion endpoint calculated for this analysis was the same as the "any lesion" category calculated by Horness et al. (1998) and Johnson et al. (2002).

Data Selection and Pairings: Liver lesion and sediment chemistry data are available from several urban areas and cleanup sites within Puget Sound. Data sets were selected for this analysis based on the following factors: 1) acceptable temporal agreement between lesion and sediment data; 2) lack of potential interactive effects with PCBs where those compounds have been identified as the primary chemical of potential concern; and 3) sufficient sediment sampling data within the estimated home range area surrounding the trawl locations. As discussed later, a minimum of 20 sediment samples are typically needed to provide statistically reliable estimates of an average HPAH exposure within a specific sampling area. This statistical requirement was most often the limiting factor determining acceptability of potential data pairs. Those sites shown in Figure 1 provided data that met the selection factors. Of the data available at the locations, pairings of sediment chemistry and liver lesion sampling events were identified that were the closest in timing to estimate exposure to fish collected within a given trawl area.

For the Eagle Harbor Superfund Site, 1991 lesion data (WDFW 2002) were paired with 1988 sediment chemistry data (CH2MHill 1989) to provide a data pair representative of conditions prior to sediment cleanup, which was initiated in 1994. As discussed in CH2MHill (1989), sediment chemistry conditions within Eagle Harbor were stable prior to cleanup. Although post-cleanup lesion data were collected by NOAA and/or WDFW at least annually following cleanup (exhibiting a steady decline over the 5-year period of record; Myers et al. 2000), and sediment data were collected in 1997 and 1999, only the 1999 data pair (WDFW 2002; Striplin 2000; PSAMP 2001) had sufficient sediment sampling data to support reliable statistical evaluations. The 1997 sediment chemistry data set were relatively limited (13 data points; SAIC 1998), and were not sufficient for an adequate statistical evaluation (see below). Both the pre- and post-cleanup pairs in Eagle Harbor have a high level of temporal agreement.

For the Hylebos Waterway (Commencement Bay Nearshore/Tideflats Superfund Site), lesion data and sediment chemistry data were all collected between 1994 and 1996 as part of the concurrent Hylebos Waterway Injury Assessment and Pre-Remedial Design (Collier 1997; SEDQUAL 2002). This pair has a high level of temporal agreement. Liver lesion data collected by WDFW (2002) at the mouth of the Thea Foss Waterway (also within the Commencement Bay Nearshore/Tideflats Superfund Site) were paired with sediment data reported by Hart Crowser (1999) in the Thea Foss Round 3 Pre-Remedial Design Assessment. Both sampling efforts occurred in 1997 and have a high level of temporal agreement.

Liver lesion data for fish collected from Elliott Bay from 1984 to 1986 (NOAA 1998) were paired with sediment chemistry data collected from 1983 to 1988 (SEDQUAL 2002). Liver lesion data for fish collected from Elliott Bay from 1989 to 1994 (NOAA 1998; WDFW 2002) were paired with sediment chemistry data collected from 1990 to 1992 (SEDQUAL 2002). In general, there was good temporal agreement for the Elliott Bay lesion-sediment chemistry pairs.

Background Sites: Background/reference areas in Puget Sound identified for NOAA's analysis (Horness et al. 1998; Johnson 2001; Johnson et al. 2002) and used in this study include Carr Inlet, Nisqually Delta, and Colvos Passage. All of the background liver lesion/sediment chemistry pairs have acceptable temporal agreement, but because of the limited number of sediment samples, there is more uncertainty in the exposure estimates within these areas. However, the calculated regression threshold values (see below) were relatively insensitive to the assumed background/reference area HPAH concentration. In addition, the resulting background lesion prevalence generated by this data set is consistent with that predicted by the larger West Coast data set in Horness et al. (1988). Thus, the limited background/reference area sediment quality data were determined to be sufficient for this analysis.

NOAA collected liver lesion and sediment chemistry data at the Nisqually Delta regional background site as part of the National Benthic Surveillance Program (NOAA 1998). The average sediment HPAH concentration reported by NOAA was paired with the liver lesion data collected by NOAA in 1984, 1985, 1986, 1990 and 1991. WDFW collected liver lesion data in 1993 and from 1995 to 1999. The WDFW data were paired with the average sediment HPAH concentrations measured by PSAMP (2001) in 1998.

Liver lesion data were obtained from fish collected in 1994 and 1995 from another regional reference site - Colvos Passage as part of the Hylebos Waterway Injury Assessment (Collier 1997). These data were paired with the average sediment concentration of HPAH measured by PSAMP (2001) in 1998. Liver lesion data for fish collected from Carr Inlet in 1996 were paired with the average sediment concentration of HPAH measured by PSAMP (2001) in 1998.

Area-weighted Exposure Estimates

Home Range Estimates: English sole are benthic feeders and therefore, must move across a forage area to meet their energetic requirements (Lassuy 1989). Although adults in Puget Sound generally move to spawning grounds annually, English sole show site fidelity and homing behavior (Day 1976, Lassuy 1989). Lassuy (1989) noted that, "most authors have concluded that movement is largely restricted to seasonal migrations in geographically segregated stocks." Based on the fact that lesion development takes several years; sampling locations in Puget Sound, some only several kilometers apart, have demonstrated different lesion prevalence; and sites that have had consistent PAH concentrations over time have also shown relatively consistent lesion prevalence; it appears that English sole are returning to the same sites after their seasonal spawning migrations. Because they apparently do move during their daily foraging activities, some home range estimate is needed to determine an exposure estimate. The home range value was selected based on a range of considerations as summarized below:

- Day (1976) conducted mark/recapture studies in central Puget Sound. Typical of other studies, there were some that strayed to other locations. But based on the typical movements of the recaptures, English sole tended to remain or return to a single area defined by Day (1976) as on the order of 5 to 10 km.

- Based on a detailed review of the available data on English sole life history, a 9 km^2 home range (approximately 1.7 km radius) estimate for English sole was used in Puget Sound to develop sediment disposal regulations for open-water areas (PSDDA 1988). The agency documents describe this value as a conservatively low estimate of the normal home range area for bottom fish in open-water regions.

- The typical trawl length used by WDFW and NOAA for collection of English sole in Puget Sound was on the order of 0.5 to 1.0 km, so that if subpopulations existed within this distance, they would be effectively composited within the trawl.

- The sites evaluated herein (especially the Superfund sites) are primarily embayments. The spatial layout of these areas likely influences the movement of fish within the waterways such that the home range may be restricted relative to open water areas. Consistent with such site characteristics, NOAA has reported differences in the prevalence of liver lesions within the Hylebos Waterway at trawl locations separated by approximately 1 km (e.g., between the Upper and Lower Turning Basins; Collier et al. 1997).

Based on the information summarized above, the typical resident area home range of English sole in Puget Sound waters is suggested to be approximately 2 km radius or less. However, considering that differences in lesion prevalence have been seen with only 1 km separation and that trawls effectively composite fish over at least 0.5 km, a 1 km radius around the centroid of the trawl was assumed in this analysis to provide a more reasonable, and likely conservatively small estimate of the home range of English sole within the embayments sampled. Where upland or intertidal zones bounded the home range circle, the smaller subtidal area within the 1 km boundary defined the home range.

Area-Average Exposure Calculation: As is the case with most environmental chemistry data, sediment quality data collected within nearly all areas of Puget Sound exhibit a pronounced skew approximating a lognormal statistical distribution, particularly within higher concentration urban areas and Superfund sites (SEDQUAL 2002). Because a log-normal distribution has a significant majority of the sampled concentrations below the calculated overall average for that distribution, a random sediment sample collected with these areas is likely to underestimate, often by a substantial margin, the true average concentration within the area. In fact, given the variability in HPAH concentrations typically observed between replicate sediment samples in Puget Sound (coefficient of variation of often exceeding 150 percent), approximately 20 samples are needed when sampling such a distribution to ensure the estimate of the average is within 50 percent of the true average concentration (p=0.05). In contrast, if only three samples are collected, the estimate the average concentration would only ensure the estimate was within 150 percent of the true average concentration (p=0.05). That three-sample average would also have a high probability of underestimating the concentration. Another way to consider this problem is in the ability to separate any two sample results as being different. Given the observed small-scale (less than 0.5 km distance) spatial variability observed within representative embayments such as Eagle Harbor and the Hylebos Waterway (coefficient of variation of approximately 160 percent), at least 20 independent sediment samples would be required to detect a 50 percent difference in mean sediment HPAH concentrations between locations (alpha=0.05, beta=0.25). For example, use of only three samples to define sediment exposure in the Hylebos waterway would only have a 150 percent or greater difference in mean sediment HPAH concentrations to be detected between locations. With only three samples per trawl location, only sites greater than 150 percent difference could be said to have different PAH concentrations.

Thus, for the purpose of this analysis, a minimum of 20 sediment samples were determined necessary to reasonably estimate HPAH exposure within specific embayments. This minimum sample size criterion was applied to the analysis presented herein.

Since it is unknown how much time an individual English sole may spend foraging in a portion of its home range, we assumed that movement and time were equitably distributed across the entire area. An area-weighted average HPAH concentration was used to estimate the long-term exposure within the home range. Thiessen polygons provide a reasonable method of estimating chemical concentrations within and between sediment sampling stations, and are routinely used to investigate contaminated sites under Ecology's SMS. They can be used to calculate the area-weighted average of a concentration over any identified area of interest. In this case, Thiessen polygons were used to generate an area-weighted average for the home range area centered on each trawl centroid. Shoreline and bathymetric details were obtained from surveys provided by the Washington Department of Natural Resources and WDFW. Since English sole primarily reside within the subtidal (versus intertidal) environment (Day 1976), the home range boundary only included the subtidal areas of the embayments. All the sediment samples collected within the home range from the subtidal zone were used for this analysis (Figure 2). For locations where the number of data points provided adequate spatial coverage of the home range area (i.e., 20 or more data points), area-weighted average concentrations were calculated using GIS software (ArcView 3.2).

In some of the embayments and during certain sampling periods, the HPAH concentrations exceeded the Apparent Effects Threshold (AET) for the benthic community. The concentrations predict effects to the benthic community and it is unknown if it could affect English sole or elicit avoidance behavior from the fish. For the purposes of this study, it was assumed that probable benthic effects occurring above the benthic AET concentration could potentially affect foraging in the area. Therefore, these areas were excluded from the home range at the site and those stations were dropped from the calculations. The result will be to produce a conservatively low estimate of the area-weighted sediment concentration

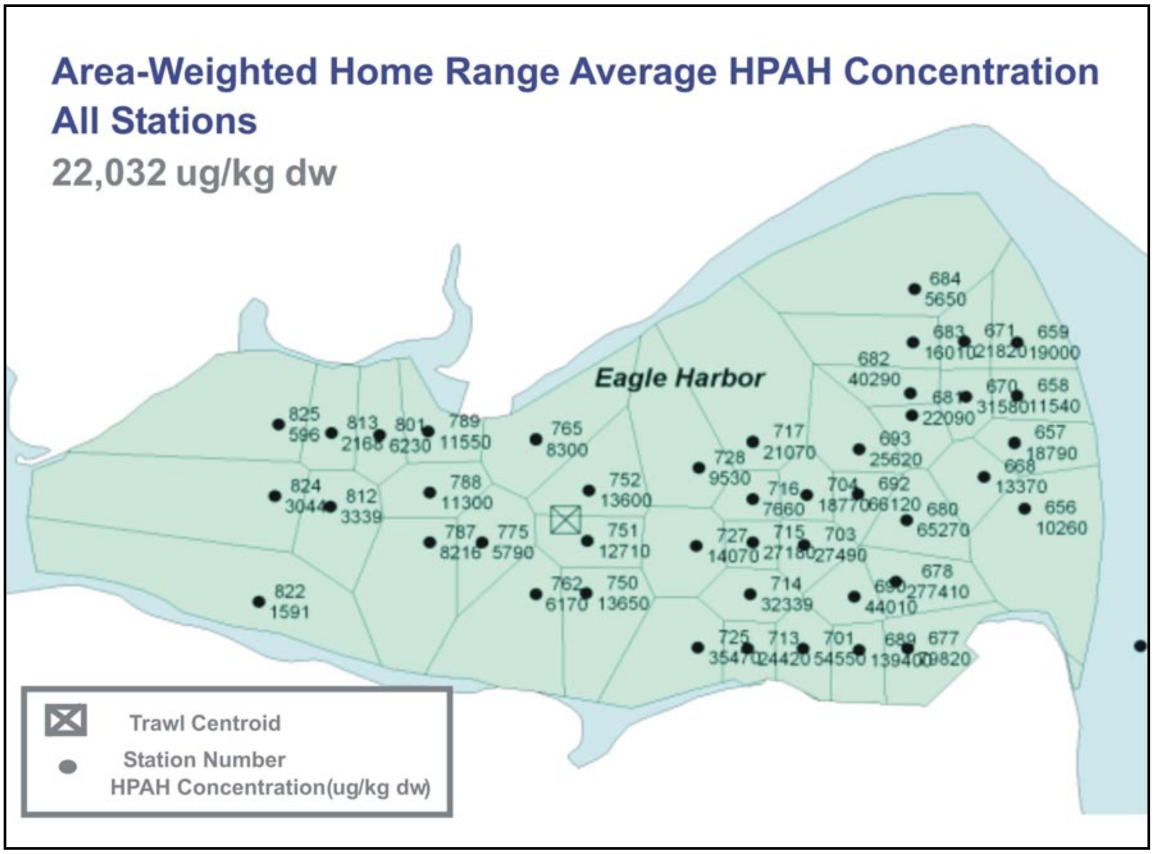

Figure 2. 1 KM radius home range area-average calculation from Eagle Harbor (pre-cleanup 1988-1991 sediment chemistry data).

for sites that have stations exceeding the benthic AET. This affected the calculation for the Eagle Harbor data pair from before the cleanup and is shown in Figure 3 (which can be compared to the previous figure before the data was excluded). Also affected was the Elliott Bay 1983-1988 data pair (not shown).

There were only three variations from the general methods described above. For the Eagle Harbor 1999 monitoring data set, five data points for the inner harbor were included from the earlier (1988) Eagle Harbor data set to provide adequate spatial coverage of the home range. These five data points are well beyond the Eagle Harbor remediation area, and are located within an area where sediment chemistry conditions were also stable prior to cleanup (CH2MHill 1989). Recent (though limited) sediment sampling by EPA and NOAA has confirmed the stability of HPAH concentrations within this area of Eagle Harbor, supporting the validity of this assumption. The Elliott Bay 1983-1988 sediment average was calculated with 17 samples following the removal of stations with HPAH exceeding the benthic AET. Also, Hart Crowser (1999) presented data for the mouth of the Thea Foss as an arithmetic average value, and their estimate was used in this analysis in lieu of more detailed Thiessen polygon determinations due to lack of enough detail in the report to generate the polygons.

Non-linear Regression Analysis

Non-linear regression analysis of liver lesion and sediment chemistry data was conducted using the hockey stick regression method outlined by Horness et al. (1998) using JMP Version 4 software. The hockey stick regression consists of two segments, a background segment with a slope of zero and a linear regression line fitted to the data above a calculated threshold value (Equation 1).

Effect = Background	For SC ≤ SCt	(Equation 1)
Effect = Background + Slope x (SC-SCt)	For SC > SCt	(Equation 2)

where SC is the sediment concentration and SCt is the sediment concentration threshold point.

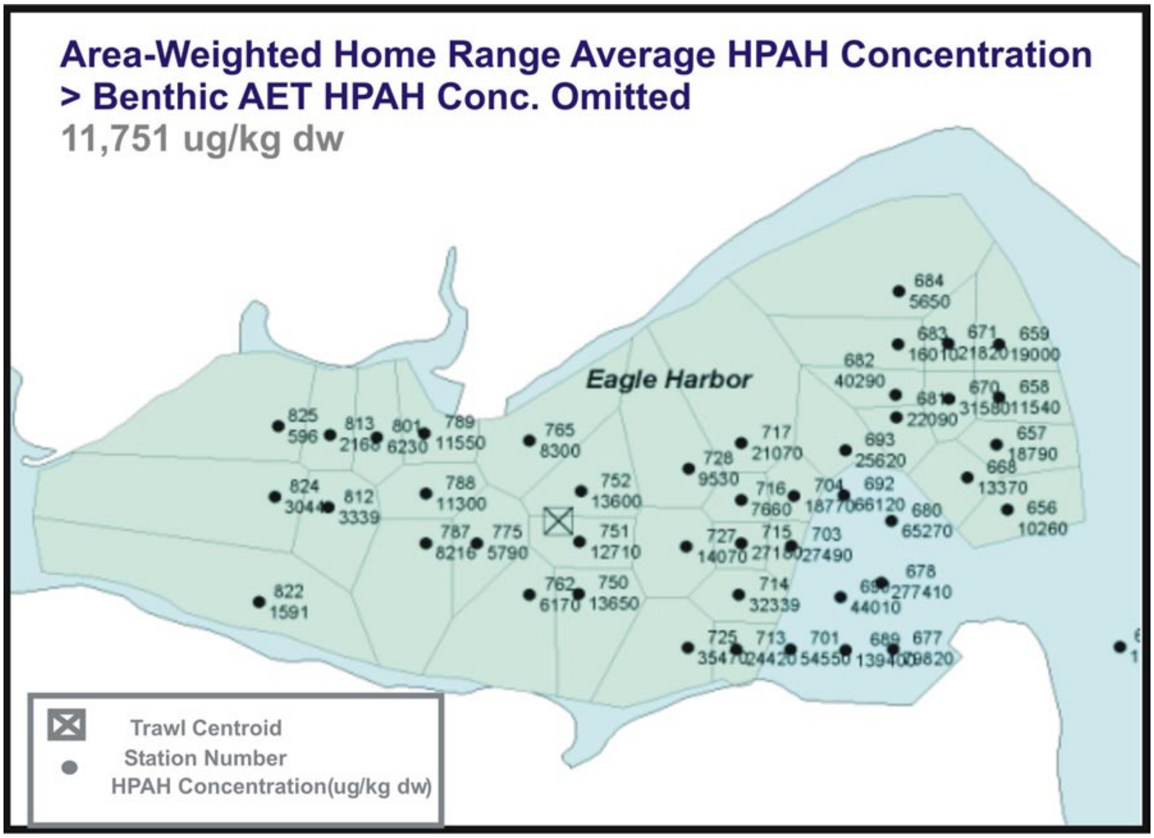

Figure 3. Revised 1 KM radius home rage area-average calculation from Eagle harbor (pre-cleanup data with HPAH concentrations greater than the benthic AET for HPAH omitted).

Consistent with the method used by Johnson et al. (2002), the regression calculation performed for this analysis estimated three parameters, the background intercept, the threshold intercept, and the slope of the regression line. The sediment concentration (log-transformed) was the dependent variable and the lesion prevalence data (untransformed) was the independent variable.

Results

Using the compiled Puget Sound database described above, the sediment HPAH concentration threshold estimate for the "any lesion" endpoint was calculated at 2,730 µg/kg dry weight (confidence interval [CI]: 1,410 to 3,770). The estimate for background incidences of liver lesions was 2.7 percent (CI: 0 to 6 percent). The regression analysis is plotted in Figure 4.

The estimated total sediment PAH threshold value for the "any lesion" endpoint presented by Horness et al. (1998) and Johnson et al. (2002) was 620 µg/kg dry weight (CI: 300-1,000), including both low and high molecular weight PAH compounds (National Benthic Surveillance Project [NBSP] list; Johnson et al. 2002). The background lesion prevalence level was 2.4 percent (CI: not reported). The background level corresponds closely with the 2.7 percent (CI: 0-6) reported in this study. The equitable background lesion prevalence levels would be expected due to the insensitivity to the lower PAH concentrations, but does suggest comparability between liver lesion data sets used for the two studies. Since the average HPAH (SMS list) to total PAH (NBSP list) ratio in sediments from these Puget Sound sites is approximately 0.85, the comparable HPAH threshold estimated by the NBSP data would be approximately 530 µg/kg dry weight. Thus, the initial sediment HPAH threshold estimated by the NOAA analysis is more than four times lower than that derived from the more rigorous analysis presented herein. This is largely attributable to differences in sediment concentration estimates between the two approaches (i.e., the 3 trawl-line samples used by Horness et al. [1998] versus the 20+ home-range samples used herein). The pronounced lognormal statistical distribution of the sediment HPAH data leads to a condition where small sample sizes will likely considerably underestimate the true average sediment HPAH concentration at a location.

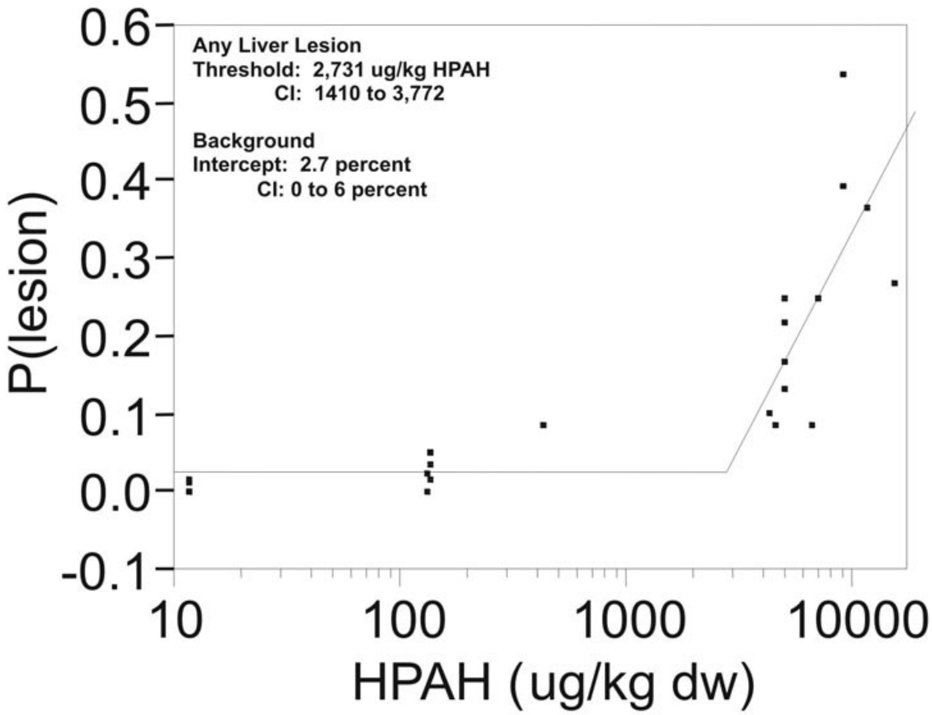

Figure 4. Hockey stick regression of hepatic lesion prevalence in English sole (any lesion) and area-weighted home range average HPAH concentrations µg/kg dry wt).

The other significant difference between NOAA's analysis results and those described herein is the inclusion of lesion and sediment quality data pairs from Puget Sound sites that were not incorporated into NOAA's initial analysis. The pre- and post-sediment cleanup data sets collected at the Eagle Harbor Superfund Site are particularly important in this analysis, as they provide field-scale experimental data that can be used to test the presumed cause-and-effect relationship between PAH exposure and liver lesion development under reasonably controlled conditions (i.e., before and after site remediation implemented by EPA beginning in 1994 to curtail sediment PAH exposure to biota).

The progressive and highly significant reduction of liver lesions in the Eagle Harbor English sole population following sediment cleanup is documented in Myers et al. (2001) and WDFW (2002). Prior to cleanup (1991 sampling), the prevalence of liver lesions in Eagle Harbor English sole averaged approximately 37 percent, among the highest observed throughout the Puget Sound region, consistent with the relatively high sediment HPAH concentrations within this site. Four to five years after EPA completed its primary sediment cleanup action, achieving an area-weighted average HPAH concentration of approximately 4,000 µg/kg dry weight within the home range area, the average incidence of liver lesions in Eagle Harbor had dropped steadily and dramatically to at or below 10 percent, which is close to the Puget Sound background/reference range calculated using non-linear regression methods (Figure 5). The mean age of fish collected prior to cleanup was 7.7 years, while the post-cleanup sample set averaged 6.2 years old. Thus, while some age-related influence may be present in these data sets, the observed difference in age can only explain a small portion of the observed decline in lesion incidence. The improvement in flatfish health in Eagle Harbor provides an especially compelling demonstration of the relationship between sediment HPAHs and liver lesions.

Discussion

Sediment chemistry stations that were located along the trawl lines were used to compare the area-weighted averages to the arithmetic averages resulting from trawl-line only samples. The data used by Horness et al. (1998) and Johnson et al. (2002) were synoptically collected with sediment chemistry data from the ends and center of the trawl line. As noted above, this type of sampling design is likely to underestimate exposure. Example calculations were performed to illustrate this sampling artifact, and are summarized below.

WDFW collected English sole from a trawl line that ran within the center of Eagle Harbor, near the ferry terminal and maintenance yard areas (see Figure 3 for trawl centroid). The trawl line is approximately centered over one PSAMP

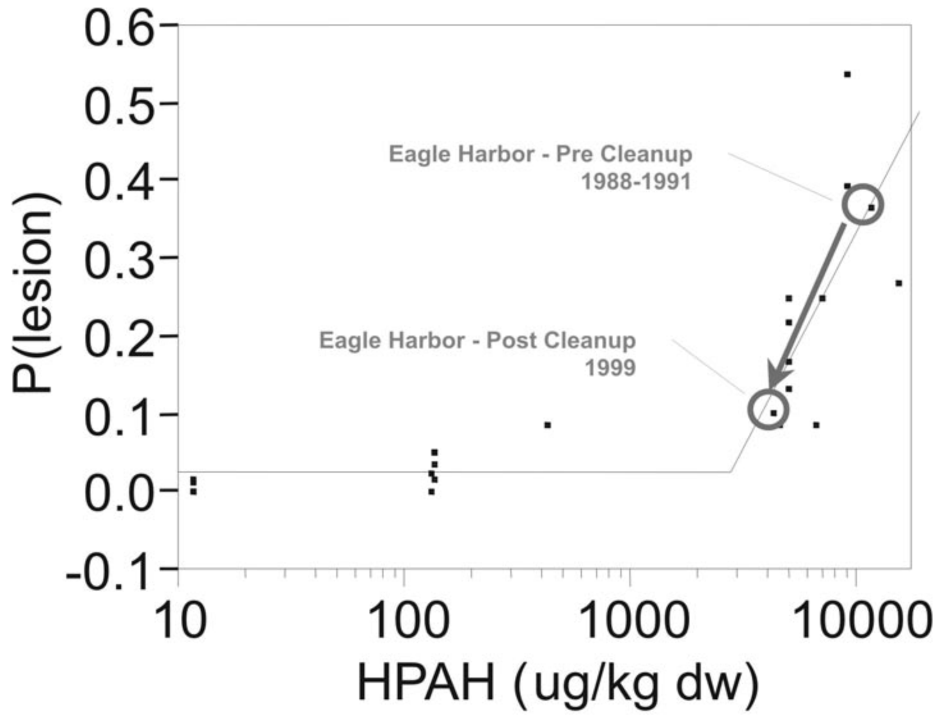

Figure 5. Eagle Harbor cleanup effect on hepatic lesion prevalence in English sole.

sediment station (station 30) so that station provides the synoptic sediment chemistry data for the trawl. The HPAH concentration for the station was 1,660 µg/kg dry weight. By comparison, the area-weighted average home-range sediment HPAH concentration for this same data set (44 sediment samples) was approximately 11,700 µg/kg dry weight. To illustrate the issue of heterogeneity, PSAMP sampled that station 5 years running, from 1989-1993, prior to the cleanup. The range of those samples was from 1,664 µg/kg to 4,120 µg/kg dry weight, with an average of 3,190 µg/kg dry weight. By coincidence, the synoptic sample was the lowest of the measurements but is the data point tied to the lesion prevalence data in the WDFW data set. The area-average home range estimate is seven times higher than the synoptic data and more than three times higher than the long term average. To approximate the three-sample method used at the other trawl sites, we took the three samples from the Eagle Harbor pre-remediation sediment data points that fell approximately within the trawl area included stations 751, 775, and 787 (CH2MHill 1988; WDFW 2002; Collier and Myers 1997; Figure 3). The arithmetic mean sediment HPAH concentration at these three stations was 6,599 µg/kg dry weight. This demonstrates that additional samples improve the estimate of the average concentration in the embayment. But the area-average home range estimate is still almost two times higher than the trawl line estimate in this situation. This pattern is repeated at the other locations, with the area-average home range estimate ranging from 50 percent to five times the trawl line estimate. Based on the stronger statistical basis supporting the area-average estimates presented herein, they provide a much more accurate estimate of the time-averaged concentration English sole are exposed to during their seasonal residence.

The Eagle Harbor data also document that sediment remediation to an area-weighted average somewhat higher than the estimated hockey stick regression threshold of 2,730 µg/kg dry weight (in this case to approximately 4,000 µg/kg dry weight) reduced liver lesion incidence to near background/reference levels. This study has provided an argument that the home range average sediment HPAH concentration provides the most representative measure of PAH exposure to bottomfish, considering relevant and appropriate statistical, behavioral, and toxicological characteristics. However, sediment remediation is often targeted to the highest concentration ("hot spot") areas of the site to accomplish the desired control of PAH exposure to bottomfish and eliminate predicted effects to the benthic community. For example, EPA's 1994 remedial action in Eagle Harbor achieved a sediment cleanup action level for individual HPAH samples of approximately 17,000 µg/kg dry weight, which corresponds generally with the SMS standard based on the lowest AET (Figure 6). Following remediation, maximum sediment HPAH concentration at the Eagle Harbor site have been maintained below this action level. Consistent with pre-construction feasibility study estimates (CH2MHill 1989), sediment cleanup to the benthic effects level reduced the home range average sediment HPAH concentration within the harbor approximately 5-fold to about 4,000 µg/kg dry weight, and concurrently reduced the incidence of liver lesions

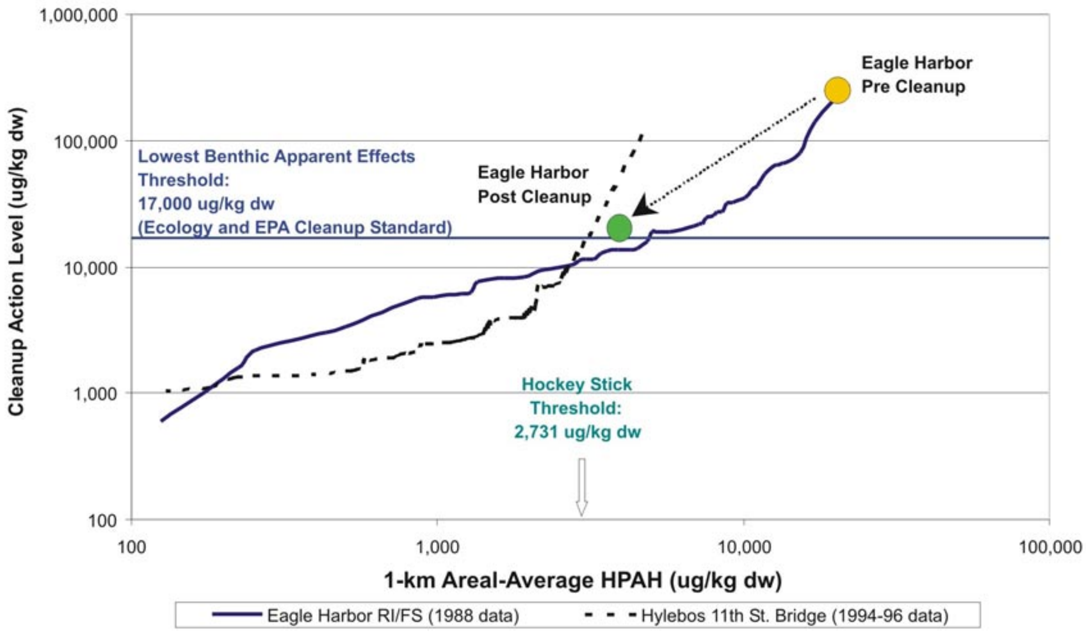

Figure 6. Relationship between sediment cleanup action level and predicted home range average HPAH concentration.

close to background/reference levels. Thus, in the case of Eagle Harbor, sediment cleanup successfully restored the incidence of liver lesions in bottomfish almost to baseline levels. Sediment remediation targeted to removing benthic effects from HPAHs may provide an appropriate level of protection from increased liver lesions in bottomfish.

The use of area-averaging of sediment concentrations across the home range provide more accurate exposure estimates that can then be used in threshold calculations. Use of only three sediment samples collected from the trawl-line do not provide an accurate estimate of sediment exposure within the home range area. By incorporating available life history information and simple bioenergetic considerations for prey needs, a reasonable home range can be constructed for English sole. By identifying a target area instead of a trawl line, the size from which sediment chemistry data can be collected and compiled is increased. This effectively increases sample size and significantly decreases the error associated with estimating the sediment concentration (in this case study from more than $\pm$150% to less than $\pm$50%). The home range concept also allows an estimation of an average exposure over time for the fish subpopulation seasonally resident at the site. Thiessen polygons are a useful tool for making area-average estimates because they provide a spatially explicit way to calculate exposure across a home range. Other spatially explicit methods for determining home range exposures are also available (e.g., inverse weighting, kreiging, etc.), although their application is not as well developed or recognized under current Puget Sound regulatory programs.

In theory, improving the ability to estimate exposure should not change the underlying dose-response relationship. Incorporating these changes into the non-linear hockey stick regression model to predict a threshold concentration where hepatic lesion prevalence should increase above background levels produces the same relative fit, confirming the underlying relationship. In fact, incorporating the exposure refinements discussed above improves the relative mean standard error for the regression from 0.12 (from the original data, Horness et al. 1998) to 0.076 in this study. The improvement in fitting the relationship provides another line of evidence that the home range approach provides a better exposure estimate. It also suggests that the four times higher threshold concentration is a more accurate reflection of HPAH exposures above which significant changes in lesion prevalence begin to occur. It should be noted that the actual threshold number is very sensitive to outliers at lower concentrations, suggesting that collection of additional data in these moderately contaminated areas would help to further refine the dose-response relationship.

Taken together, the results also suggest that if this dose-response relationship is to be considered as a sediment benchmark for effects to English sole, then the area-weighted home range exposure estimate approach should be used. Puget Sound areas where English sole have been collected and analyzed, and for which extensive sediment PAH data are available, provide the largest relational liver lesion/sediment chemistry data set. As we have shown, using synoptic data is not required for calculating such benchmarks, provided that an appropriate level of recency is attained. The relationship between sediment associated PAHs and liver lesions in English sole can also be better understood by incorporating the highly relevant pre- and post-sediment cleanup data sets collected at the Eagle Harbor Superfund Site. The Eagle Harbor data sets provide field-scale experimental data that can be used to test the presumed cause-and-effect relationship between PAH exposure and liver lesion development under reasonably controlled conditions (i.e., before and after site remediation implemented by EPA to curtail sediment PAH exposure to biota). The validation of the model fit using Puget Sound data suggests that the results may potentially be applicable to other comparable areas.

In summary, this study illustrates how incorporating the concept of home range exposure, along with inclusion of other available data sets, substantially changes the outcome of a sediment threshold concentration. The initial sediment HPAH benchmark estimated by Horness et al. (1998) and Johnson et al. (2002) is more than four times lower than that derived from the more statistically rigorous analysis presented herein. Given the magnitude of the apparent difference in results between the two approaches, and the stronger statistical basis supporting the area-average estimates presented herein, the initial threshold concentration estimates provided in Horness et al. (1998) are associated with considerable uncertainty, and are likely biased low. This analysis did confirm the dose-response relationship modeled by the non-linear hockey stick regression but also demonstrated that area-weighted home range exposure estimates will provide a more reliable exposure estimate and resulting threshold concentration. Any sediment benchmarks should be calculated using the area-weighted home range exposure estimates. The work summarized herein also suggests that, for many sites, sediment cleanup of individual "hot spot" areas to achieve benthic protection will likely also successfully restore the incidence of liver lesions in bottomfish to baseline levels.

Based on the analysis summarized above, further research is likely needed in several areas prior to attempting to use this dose-response relationship to develop sediment benchmarks. Refining the prediction of English sole's home range, adding sites as more data pairs become available, assessing different PAH groupings in the regression and their varying potency relationship to the assortments found *in-situ*, and further evaluating bioavailability and age effects all will improve our understanding of the true dose-response.

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